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2920-Pos Board B612

Quantitation of Cell Wall Growth Suggests Feedback Mechanisms that Robustly Build Rod-Like Bacteria

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In bacteria, a host of enzymes regulates the reproducible and robust construction of the cell wall, whose mechanical integrity is crucial for viability under osmotic stress. Antibiotics that target these enzymes disrupt cell wall construction, ultimately leading to mechanical failure of the cell. Our work explores the physical mechanisms of cell growth and death, as a guide to understanding antibiotic mechanisms that disrupt mechanical properties of the cell. We use a combination of cell wall fluorescent labeling, high resolution time-lapse microscopy, and computational image processing to characterize where, and with what dynamics, cell wall and outer membrane growth occurs. When cell-shape analysis is combined biophysical simulations of growth, our data strongly suggest that dynamic localization of the bacterial MreB cytoskeleton is part of a curvature sensing and growth feedback mechanism that orchestrates heterogeneous growth to maintain rod-like shape and regulate mechanical stress. Analysis of MreB and cell-surface marker fluorescence indicates that the cytoskeleton is present at sites of active growth and that chemical depolymerization of the cytoskeleton causes homogenous, unstructured growth and eventual cell death by rupture. Quantitative tracking of growth is an effective method for characterizing cell wall mechanical failure, and these techniques pave the way for studying the detailed dynamics of growth-associated proteins and their disturbance by antibiotics.

2921-Pos Board B613

Surviving a Bumpy Ride in the Oropharynx: Bacterial Pili as Nano-Seatbelts that Dissipate Mechanical Energy

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Bacterial pili function in cellular adhesion, and must withstand large mechanical stresses in host environments, such as coughing and chewing. In gram positive bacteria, pili are covalently-linked polymers of single protein subunits, termed pilins. Gram positive pilins uniquely possess intramolecular isopeptide bonds that bridge the peptide backbone to form bypass force transduction pathways. In the crystal structure of Spy0128, a pilin from *Streptococcus pyogenes*, isopeptide bonds link the N- and C-terminal β-strands. Consequently, Spy0128 is mechanically inextensible. Here we report on the mechanical properties of two related pilins, SpaA from *Corynebacterium diphtheriae* and FimA from *Actinomyces oris*, using atomic force microscopy (AFM)-based single molecule force spectroscopy. In the crystal structures of SpaA and FimA, the isopeptide bonds do not directly link the N- and C-terminal β-strands in a single pilin domain. Instead, the isopeptide arrangement creates a ~40 residue polypeptide loop that resembles a slackened seatbelt, which we predict is sensitive to mechanical unfolding. We find that both SpaA and FimA extend to 14 nm under mechanical force, consistent with our structure-based prediction of unfolding of the "nano-seatbelt" from a slackened to a taut conformation. At a loading rate of 400 nm/s, these loops unfold at forces of ~503pN in SpaA and ~665pN in FimA; as such, SpaA and FimA are among the most mechanically stable proteins yet reported. When the force perturbation is removed, the loops refold at a rapid rate of 29 s^{-1} or higher**.** Remarkably, the mechanical stabilities are ~75pN weaker upon refolding, suggesting that gaining full mechanical stability requires maturation. The high mechanical stability and rapid refolding of the nano-seatbelts suggest a mechanism whereby pilin subunits, polymerized as tens-to-hundreds of repeats in pili, readily absorb and recover from mechanical shocks.

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Pressure-Speed Relationship of the Sodium-Driven Flagellar Motor of Vibrio Alginolyticus

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The bacterial flagellar motor is a molecular machine that converts an ion flux to the rotation of a helical flagellar filament. Motor rotation rate and directions can be changed by environmental factors such as temperature, pH, and solvation. Hydrostatic pressure is also an inhibitor of the rotation of flagellar motors [1, 2]. Our previous results indicated that the application of pressure inhibits the rate of ion tranlocation in the mechanochemical energy translation, but the detailed mechanism is still unknown. Here, we characterized the pressure dependence of the rotational speed of sodium-driven flagellar motor in swimming *Vibrio alginolyticus* cells. The motor in strain NMB136 exclusively rotates in counter-clockwise direction and propells the cell body forward. We monitored the pressure-induced effects on the behavior of the cells that swim freely in solution. The swimming speed exponentially decreased with the increment of pressure. The sodium concentration dependence of the swimming speed at each pressure was well described by a Michaelis-Menten kinetics. The applied pressures decreased the maximum velocity, but increased the Michalis constant. Our results showed that the motor has at least two pressure-sensitive reactions, one of which is the binding process of external sodium ions to the motor. Another is the post-sodium-binding process, suggesting sodium transit and/or its release to inside the cell.

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2923-Pos Board B615

Motility Enhancement through Surface Modification is Sufficient for Emergent Behaviors During Phototaxis

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The emergent behaviors of communities of genotypically identical cells cannot be easily predicted from the behaviors of individual cells. In many instances, direct cell-cell communication or cell differentiation play important roles in the transition from individual to community behavior. In the cyanobacterium Synechocystis, cells exhibit light-directed motility (phototaxis). This process occurs at both single-cell and community scales. While single cells undergo a biased random walk, an inoculation of cells on an agarose surface can be observed to form dynamic finger-like projections toward a directed light source. These subcommunities consist of a high concentration of cells concentrated at the progressing front, followed by a lower concentration of cells distributed along the finger. Results from time-lapse microscopy suggest that cells secrete an extracellular polymeric substance (EPS) that modifies the physical properties of the substrate, leading to enhanced motility and the ability to detect tracks left by other cell groups. Our quantitative, single-cell tracking results show that the EPS confers no information of directionality or memory of light directionality, suggesting its major role in motility enhancement. Furthermore, the distribution profiles of the movement bias of single cells vary spatially across the inoculation, with cells in finger-like projections having a more pronounced movement bias toward light. We have developed a cellular automata model that demonstrates that indirect, surface-based communication conferred by EPS is sufficient to create distinct motile groups whose shape and bias distributions match our experimental observations, even in the absence of direct cellular interactions or changes in single-cell behavior. Therefore, our modeling and experiments provide a framework to show that the emergent behaviors of phototactic communities involve modification of the substrate, and this form of surface-based communication could provide insight into the behavior of a wide array of biological communities.

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High throughput 3D Palm Imaging Elucidates Mechanisms of Bacterial Cell Division

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We created a high throughput modality of photoactivated localization microscopy, HTPALM, which enables automated 3D PALM imaging of hundreds of synchronized bacteria during all stages of the cell cycle. We used HTPALM to investigate the nanoscale organization of the bacterial cell division protein FtsZ in live C. crescentus. We observed that FtsZ predominantly localizes as a patchy mid-cell band, and only rarely as a continuous ring, supporting a model of "Z-ring" organization where FtsZ protofilaments are randomly distributed within the band and interact only weakly. We found evidence for a previously unidentified period of rapid ring contraction in the final stages of the cell cycle. We also found that induction of the SOS response produced high-density continuous Z-rings which may obstruct